Insights into the Pathogenesis, Virulence Factors, and Diagnosis of *Helicobacter pylori*: A Comprehensive Review

Ali Saad Kadhim[1], Abdullah Salim Al-Karawi[2*]

ABSTRACT

The *H. pylori* bacterium, which resides in the human stomach and is linked to a number of gastrointestinal illnesses, is introduced in-depth in this article. We examine how *H. pylori* establishes infection, thwarts the hosting immune response, and causes inflammation as we explore the pathophysiology of this bacterium. We also talked about how *H. pylori* influences host cells and develops a favorable environment for survival. We also go over the many methods for identifying H. pylori, such as endoscopy, biopsy, blood, breath, feces, and others. We stress the importance of an accurate and speedy diagnosis while outlining the advantages and disadvantages of each diagnostic strategy to manage disorders caused by *H. pylori* effectively. Throughout this review, we aim to unravel the mysteries surrounding H. pylori, providing valuable insights into its pathogenesis, the intricate interplay of virulence factors, and the diverse diagnostic strategies employed in clinical practice. Enhancing our understanding of *H. pylori* can pave the way for improved therapies and patient outcomes.

INTRODUCTION

Firstly, *Helicobacter pylori* infection has become more frequent in recent years, affecting around population (Keller *et al.*, 2021; Tonkic *et al.*, 2012). Accurate *H. pylori* detection is essential for treating symptomatic people who are infected. Since Marshall and others' 1983 discovery of this spiral-shaped, gram-negative bacteria, many diagnostic methods have been created (Kim & Wang, 2021). Some tests base their results on a variety of bacterial traits, such as their appearance (histology, culture), immunology (serology, stool test, immunohistochemistry), genetics (PCR), or enzymatic activity (13C-urea breath test, quick urease test) (Mišak & Hojsak, 2021). Generally, these techniques may be divided between invasive testing (histology, urease test, culture), which demands endoscopy of the upper gastrointestinal tract and stomach biopsies, and non-invasive tests (serology, 13C-urea breath test, stool antigen test). Each test has advantages, weaknesses, and restrictions that vary depending on the clinical circumstances and the inquest (Mišak & Hojsak, 2021).

Description of *H. pylori*

Genomic characteristics, plasmid presence, and strain diversity are all pertinent factors that warrant consideration. The two sequenced genomes of *Helicobacter pylori* exhibit a size of more or less 1.9 Mbp and possess a G+C composition ranging from 31% to 45% (4). Notably, this bacteria strain 27595 encompasses 1,587 genes, while strain J99 contains a slightly lower count of 1,491 genes (Zamani *et al.*, 2018). It is deserve noting with various strains of this bacteria may harbor cryptic plasmids that lack discernible antibiotic resistance or virulence genes. Interestingly, certain plasmids are utilized in the

development of *H. pylori* - Escherichia coli shuttlecock vectors for cloning investigations (Pohl *et al.*, 2019). While the presence of *H. pylori*-infected bacterial virus has been recognized, there remains a need for comprehensive characterization in this regard. In contrast to highly clonal bacterial diseases such as Mycobacterium tuberculosis, *H. pylori* exhibits genetic diversity, indicating a lack of clonality (Leszczyńska *et al.*, 2010). Consequently, each patient harboring *H. pylori* carries a distinct strain, although minor variations may exist among relatives. The genetic variability observed in *H. pylori* may represent an adaptive response to the gastrointestinal environment of its host and the unique patterns exhibited (Leszczyńska *et al.*, 2010). Various mechanisms such as DNA rearrangement, as well as the insertion and deletion of foreign sequences, are considered to contribute to the genetic heterogeneity observed in *H. pylori* (Ferwana *et al.*, 2015).

Virulence factors of *H. pylori*
PAI cag

Although *H. pylori* infection always leads in recurrent gastritis, the majority of infected patients have absence further issues and show no evident clinical indicators of infection (Mezmale *et al.*, 2020). This raised the possibility that certain strains are more virulent than others. Early studies of *H. pylori* strains' varied pathogenic qualities revealed that increasing pathogenicity was associated into capacity of their in addition aggressive strains to generate phenotypic alterations, hyalinization, and progressive degeneration of in vitro-cultured cells. This gene is present in around 60 to 80% it (Malfertheiner *et al.*, 2017). and is a signal for a genomic PAI of about 40 kb that estimate between 37 and 51 proteins (Bessède *et al.*, 2017), depending on the strain examined. CagA+ strains

[1] Department of science, Basic Education collage, Wasit University, Wasit, Iraq
[2] Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq
[*] Corresponding author's e-mail: Abdullah.S.Shaker@uomustansiriyah.edu.iq
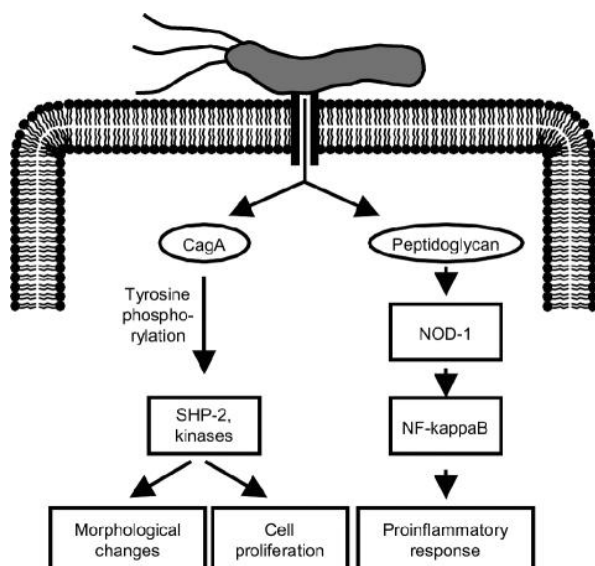
Figure 1: Roles of the Cag type IV in immunological regulation, cell multiplication, and morphologic alterations are depicted schematically.

have the cag PAI and are usually recognized in sufferer by their capability to produce important antibody titers toward the CagA marker protein (Leszczyńska *et al.*, 2010; Pohl *et al.*, 2019).

VacA Vacuolating Cytotoxin
Ninety-five kDa protein that promotes extensive hyalinization in epithelial cells is secreted by approximately fifty percent of all pylori strains (Pohl *et al.*, 2019). VacA protein involved in the etiology of peptic ulcers and gastric cancer. Notwithstanding, VacA hasn't required
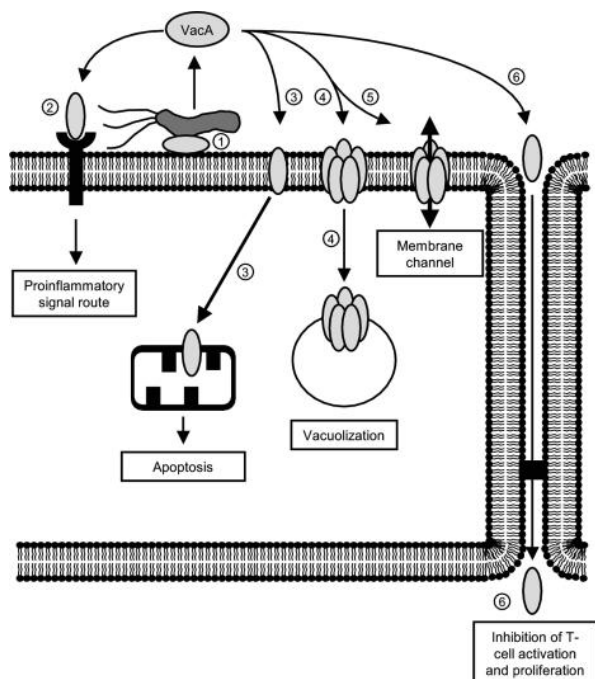

Figure 2: The VacA protein regulates cellular processes in several ways, helping in *H. pylori* chronic colonization of the stomach mucosa (6).

about *H. pylori* in vitro extension, it has been shown to contribute to *H. pylori* stomach colonization in mice significantly (Zamani *et al.*, 2018). VacA's activities include membrane channel formation, disruption of endosomal and lysosomal activity, influence on integrin receptor-induced cell signaling, interference with cytoskeleton-dependent cell functions, induction of apoptosis, and immunological control (Fig.2) (Bessède *et al.*, 2017; Nakashima *et al.*, 2018).

Acid Resistance
Although *Helicobacter pylori* is not classified as an acidophile, it possesses a noteworthy capacity to thrive in the stomach's acidic environment (Ferwana *et al.*, 2015). The pH levels within the gastrointestinal mucosa are typically believed to range from 4 to 6.5; however, instances of acid shocks can occur. Consequently, *H. pylori* necessitate protective mechanisms against critical acid shocks and the ability to adapt to pH levels of approximately 5.5. Upon entry, *H. pylori* is presumed to swiftly migrate towards employing chemotactic motility to exploit the urea and bicarbonate gradients in the stomach situation (Nakashima *et al.*, 2018). This quick migration is imperative for *H. pylori* as its motility is compromised within the acidic milieu of the stomach lumen (Malfertheiner *et al.*, 2017).

Adhesins and Outer Membrane Proteins
Numerous bacterial element influence *H. pylori*'s adhesion to the gastric epithelium. considering the bacterium's restricted horde assortment, the prevalence of adhesins likely signifies their relevance to the bacteria. However, evaluating the individual contribution of each adhesin presents significant challenges (Nakashima *et al.*, 2018). Consequently, our primary focus will be directed toward investigating the potential significance of the three Hop proteins, which possess sufficient evidence to elucidate their role in the pathophysiology of *H. pylori* infection (Tonkic *et al.*, 2018).

Methods of Diagnosis
Invasive Tests
Rapid Urease Test
An affordable, fast, and accurate test for finding *Helicobacter pylori* is the rapid urease test (RUT). In terms of sensitivity, it is nonetheless constrained. If the bacterial load in the biopsy is less than 10^4, many commercial tests might produce false-negative findings (Zamani *et al.*, 2018). Additionally, urease-positive bacteria like Staphylococcus capitis uratolytic have the potential to provide false-positive findings (Pohl *et al.*, 2019). Different commercial RUTs were evaluated in a German investigation, and it was discovered that they were all *H. pylori* detection is both sensitive and specific. [5] Amazingly, they were even more accurate than histology, particularly in individuals who had recently taken antibiotics or proton pump inhibitors (PPIs) (Leszczyńska *et al.*, 2010). The stomach biopsy used for the rapid urease test (RUT) also has the added advantage that it may be used for other assays,

such the *Helicobacter pylori* PCR (Ferwana *et al.*, 2015). In Japanese inquiries, the same RUT material was utilized for a quantitative PCR (qPCR) highlighting the EBV oriP gene because the Epstein-Barr virus (EBV) causes only a small fraction of gastric hostilities (GCs) (Mezmale *et al.*, 2020). In the 10 patients that were examined, EBV was present in 4 cases whereas *H. pylori* was present in 9 cases. This method emphasizes the possibility of examining numerous infections with the same biopsy sample in order to gain useful diagnostic data (Malfertheiner *et al.*, 2017).

Histology

Helicobacter pylori may still be seen using immunostaining or standard staining procedures in histology, which is still a common diagnostic approach (Bessède *et al.*, 2017). However, an innovation uses a fresh strategy with a fluorescent probe that can be activated by glutamyl transpeptidase (GGT) (Nakashima *et al.*, 2018). In this approach, a probe called-glutamyl hydroxymethyl rhodamine green combines with GGT to produce an instantaneous fluorescence (Bang *et al.*, 2020).The probe was used ex-vivo on stomach biopsies to assess *H. pylori*'s GGT activity. Its sensitivity, which ranges from 75% to 82%, is still rather low. Another research, which concentrated on the pathogenic features, showed how *H. pylori* affected diseases including Brunner's gland hyperplasia and duodenal hamartomas (Tonkic *et al.*, 2018).

Menetic Methods
Poly Chain Reaction (PCR)

Compared to other approaches, PCR, particularly the test that targets the 16S rRNA gene, has been shown to be a much sensible way to detect H pylori in stomach biopsies (Benoit *et al.*, 2018). The rising problem of antibiotic resistance complicates the management of *H. pylori* infection (Makristathis *et al.*, 2019). Before cultivating *H. pylori*, real-time polymerase chain reaction (RT-PCR) is being used increasingly frequently to solve this problem (Godbole *et al.*, 2020). High sensitivity and specificity, Fast response for a few hours, and comfortable transportation circumstances are only a few of the noteworthy benefits of RT-PCR. There are many commercial RT-PCR tests that have the added benefit of identifying resistance to macrolide antibiotics linked to 3 distinct genetic variation-A2142C on the 23S rRNA gene (Dalla Nora *et al.*, 2016). However, alone mercantile assay—the Genotype HelicoDR method made by German company can identify frequent SNPs in the gyrA and 23S rRNA genes (N87K, D91G, D91N, and D91Y) (Dalla Nora *et al.*, 2016; Stefano, Rosalia, *et al.*, 2018)

Advancing Genetics Method

The molecular biology discipline uses next-generation sequencing (NGS), a potent and sophisticated technique, to analyze the genetic makeup of many species, including H pylori (Pich, 2019). NGS technology enables rapid and high-throughput DNA or RNA sample sequencing, providing precise information on the genetic makeup of the organism under study (Szymczak *et al.*, 2020). NGS has demonstrated to be an effective technology in the context of *H. pylori* for a diversity of applications. It enables the detection of genetic adjusting in the *H. pylori* genome, such as integration, abolition, and structural variants (Szymczak *et al.*, 2020). Using this data, researchers may trace the spread of *H. pylori* throughout communities, study the genetic variety of various strains, and look into the development of antibiotic resistance (Nezami *et al.*, 2019). Researchers may explore the intricate connections between *H. pylori* and other microbes in the human gut thanks to NGS, which makes it easier to comprehensively analyze the whole microbial community contained in a sample. This method sheds light on the *H. pylori* infection and related disorders' effects on the microbiome's makeup and functional capabilities (Nyssen *et al.*, 2022).

NGS may also be used for proteome research, which enables the profiling of gene expression patterns in *H. pylori*. This helps in understanding the molecular processes underlying *H. pylori* pathogenesis and host-pathogen interactions (Sonnenberg *et al.*, 2020). According to a Swiss research, there is a high degree of concordance more than 99% between the findings of tests for phenotypic antibiotic sensitivity and the discovery of genetic diversity in certain genes linked to antibiotic opposition (Bair *et al.*, 2020). The 23S rRNA, gyrA, and rpoB genes, which are linked to levofloxacin, and rifampicin resistance, respectively, were examined using whole genome sequencing (WGS) (Ding, 2020). This association suggests that for these specific drugs, WGS can accurately predict antibiotic resisting in *H. pylori* strains. However, the study discovered that the presence of different SNPs in the frxA and rdxA genes could not predict metronidazole resistance, indicating that alternative processes may be involved in metronidazole resistance. The results of a study carried out in Cambodia, which showed a substantial association between genotypic antibiotic susceptibility and WGS analysis of *H. pylori* strains, were reported with similar findings (Dore & Pes, 2021; Zhao *et al.*, 2021)

Non-Invasive Tests
Urea Breath Test

Studies on urea breath tests (UBT) have been published in the previous year. One noteworthy investigation examined the accuracy of Bags for collecting breath in identifying *H. pylori*. Over 250 patients participated in the trial, which used the Breath-ID HP Lab System (Keller *et al.*, 2021). The Breath-ID HP Lab System is very accurate and has a number of improvements over the prior Breath-ID HP equipment, according to the study's authors (Makristathis *et al.*, 2019). These benefits include the capacity to examine numerous samples at once and the bags' outstanding stability, which makes transportation easier. The novel approach performed better than histology and the rapid urease test (RUT) when compared (Ford *et al.*, 2020). It is important to note that further study is needed to examine and evaluate the

results and possible advantages of the Breath-ID Hp Lab System in *H. pylori* identification and management because there haven't been many studies published on UBT in the last year (Q. Chen *et al.*, 2019). A recent meta-analysis conducted by (Chiang *et al.*, 2021) focused on the accuracy of 13C-urea breath tests (13C-UBT) in Asia (Lee *et al.*, 2021).

The results of the meta-analysis revealed that the sensitivity and specificity of the 13C-UBT exploring magnificent (Graham, 2020). However, some degree of multiformity was esteemed across the various assessments. Nonetheless, reconsideration indicated that it is feasible to reduce this multiformity by adjusting certain factors, such as the dose of urea administered and the timing of breath sample collection. The findings of this meta-analysis highlight the overall reliability of 13C-UBT as a diagnostic tool for detecting *H. pylori* infection in the Asian population (Graham, 2020). The authors suggest that standardizing the dose and collection time of breath samples could further enhance the consistency and accuracy of 13C-UBT results across different studies (Al Nabhani *et al.*, 2019).

Serology

Research was done in Japan to evaluate the diagnostic precision of two ELISA kits and two latex immunoassay kits, which make up the four commercially available assays for identifying *H. pylori* infection (D. Chen *et al.*, 2019). In Tokyo, the study's main goal was to assess how well these kits can identify *H. pylori* infection. In order to compare the diagnostic accuracy of the kits to recognized reference techniques, they were put through extensive testing and analysis (Baruch *et al.*, 2021). Seropositivity to H pylori proteins was used in research conducted mostly in the United States to identify individuals with current infection. They used multiplex serology to evaluate antibody triggering to 13 H pylori proteins in blood specimens from patients undergoing UBT (Davar *et al.*, 2021). A cutoff was used to assess sensitivity in order to obtain 90% specificity. They eventually came to the conclusion that seropositivity to at least two of the *H. pylori* proteins, including VacA, and HP1564, indicated vibrant *H. pylori* infection with high specificity and sensitivity and may enable an estimation of the incidence of active *H. pylori* disorder essentially accomplice (Begka *et al.*, 2020).

In an analysis that combined anti-H pylori antibodies with the Kyoto endoscopic score, the role of these antibodies in the development of GC was finally clarified. Scalability, emaciation and ageing among 41 and 59 years were linked to improved serum antibody titer infected individuals, according to a multivariate analysis of 874 cases (D'Amato *et al.*, 2020).

Stool Antigen Tests

The medical efficacy of the Meridian pylori faeces antigen testing evaluated in a study involving 277 individuals who underwent endoscopies. Compared to an integrated mention test comprising other tests, the Meridian H pylori SAT demonstrated an accuracy of 97.5% and a precision of 98.6%, indicating as a whole effectiveness with identifying pylori. A comparison was made between a CLIA assay and an ELISA, the R-BioPharma, and an immunochromatography test (ICT) using stool samples from 266 patients. The results obtained from both kits used for H pylori detection agreed. To assess the reliability about a novel ICT called the Vstrip® pylori faeces antigen fast test (Fumet *et al.*, 2018)And monitor the frequency about *H. pylori* in kora, researchers conducted a study involving 367 participants, such as 152 asymptomatic voluntary work and 195 instructive patients (Uribe-Herranz *et al.*, 2018; van Wijck *et al.*, 2018)

Genetics Test

A comparative scrutiny was done to evaluate the efficiency of an enhanced high-throughput, semi-automated technique in detecting and assessing clarithromycin susceptibility of *H. pylori* in unpreserved fecal and stomach specimens, as compared to a previously described manual procedure (Wilson *et al.*, 2020). DNA extraction was performed on samples obtained from 96 symptomatic patients using both the Magna Pure 96 and QIAamp Fast Stool kits in parallel (57). The results obtained from stomach biopsies and fecal samples demonstrated the feasibility of using the semi-automated method for the detection and genotyping of pylori (Zamani *et al.*, 2018). A prospective multicenter trial involving 1200 people was done to evaluate the Amplidiag® H pylori + ClariR test's capacity to detect *H. pylori* and measure clarithromycin resistance (Aguilera Matos *et al.*, 2020). To identify the *H. pylori* glmM gene and mutations in the 23S rRNA genes associated with clarithromycin resistance, DNA extracted from stools using an automatic extraction method (EasyMAG® bioMérieux) was compared to culture/E-test and quadruplex real-time PCR performed on two gastric biopsies (Htun *et al.*, 2018). However, only 160 patients (Jaka *et al.*, 2018). from the group produced valid data. After testing several published nested PCR assays that exhibited limited specificity, the authors designed a novel nested PCR assay targeting the variable regions of the 16S rRNA gene (de Martel *et al.*, 2020; Stefano, Marco, *et al.*, 2018).

Novel Endoscopic Imaging Techniques

Several attempts have been made in the past to forecast the possibility of an *H. pylori* infection during endoscopy (Poddar, 2019). Nonetheless, there hasn't been much of a relationship between reported gastritis and histology using typical white-light endoscopy. magnification with high-resolution Endoscopes with 115 times magnification and a resolution of 7.9 ml (Hooi *et al.*, 2017). The microvasculature of the body is seen below, which is made up of a honeycomb-like subepithelial capillary network produced by polygonal capillary loops that circle the stomach pits before combining into collecting venules (Park *et al.*, 2021). Chronic *H. pylori*-

associated gastritis disrupts this normal microvascular arrangement. The narrowing of the pits and the disappearance of collecting venules signify Magnification endoscopy and narrow-band imaging appear to provide accurate *H. pylori* infection prediction with an excellent degree of collaboration among observers (Hathroubi *et al.*, 2020). New endoscopic methods, including confocal endomicroscopy, focus on seeing microorganisms up close (Ierardi *et al.*, 2020).

CONCLUSION

In conclusion, a variety of considerations, including as the unique clinical environment, efficiency, the liability of getting positive test results, and the accessibility of the tests, must be taken into consideration when selecting the most suitable diagnostic test for *H. pylori* infection. These factors are essential in choosing the best strategy for correctly identifying *H. pylori* infection in a particular situation. For to choose the best diagnostic test for their patients, healthcare professionals and physicians should carefully consider these variables.

REFERENCES

Aguilera Matos, I., Diaz Oliva, S. E., Escobedo, A. A., Villa Jiménez, O. M., & Velazco Villaurrutia, Y. D. C. (2020). Helicobacter pylori infection in children. *BMJ Paediatr Open, 4*(1), e000679. https://doi.org/10.1136/bmjpo-2020-000679

Al Nabhani, Z., Dulauroy, S., Marques, R., Cousu, C., Al Bounny, S., Déjardin, F., Sparwasser, T., Bérard, M., Cerf-Bensussan, N., & Eberl, G. (2019). A weaning reaction to microbiota is required for resistance to immunopathologies in the adult. *Immunity, 50*(5), 1276-1288. e1275.

Bair, M. J., Chuang, S. L., Lei, W. Y., Chen, C. L., Tian, H. W., Chiang, T. H., Su, W. W. Y., Lin, C. C., Chung Lo, Y. T., & Jou, Y. Y. (2020). Planning mass eradication of Helicobacter pylori infection for indigenous Taiwanese peoples to reduce gastric cancer. *Journal of Gastroenterology and Hepatology, 35*(4), 609-616.

Bang, C. S., Lee, J. J., & Baik, G. H. (2020). Artificial intelligence for the prediction of Helicobacter pylori infection in endoscopic images: systematic review and meta-analysis of diagnostic test accuracy. *Journal of medical Internet research, 22*(9), e21983.

Baruch, E. N., Youngster, I., Ben-Betzalel, G., Ortenberg, R.,

Lahat, A., Katz, L., Adler, K., Dick-Necula, D., Raskin, S., & Bloch, N. (2021). Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. *Science, 371*(6529), 602-609.

Begka, C., Pattaroni, C., Mooser, C., Nancey, S., McCoy, K. D., Velin, D., & Maillard, M. H. (2020). Toll-Interacting protein regulates immune cell infiltration and promotes colitis-associated cancer. *Iscience, 23*(3).

Benoit, A., Hoyeau, N., & Flejou, J.-F. (2018). Diagnosis of Helicobacter pylori infection on gastric biopsies: Standard stain, special stain or immunohistochemistry? Annales de Pathologie,

Bessède, E., Arantes, V., Mégraud, F., & Coelho, L. G. (2017). Diagnosis of Helicobacter pylori infection. *Helicobacter, 22*, e12404.

Chen, D., Wu, J., Jin, D., Wang, B., & Cao, H. (2019). Fecal microbiota transplantation in cancer management: Current status and perspectives. *International journal of cancer, 145*(8), 2021-2031.

Chen, Q., Liang, X., Long, X., Yu, L., Liu, W., & Lu, H. (2019). Cost-effectiveness analysis of screen-and-treat strategy in asymptomatic Chinese for preventing Helicobacter pylori-associated diseases. *Helicobacter, 24*(2), e12563.

Chiang, T.-H., Chang, W.-J., Chen, S. L.-S., Yen, A. M.-F., Fann, J. C.-Y., Chiu, S. Y.-H., Chen, Y.-R., Chuang, S.-L., Shieh, C.-F., & Liu, C.-Y. (2021). Mass eradication of Helicobacter pylori to reduce gastric cancer incidence and mortality: a long-term cohort study on Matsu Islands. *Gut, 70*(2), 243-250.

D'Amato, A., Di Cesare Mannelli, L., Lucarini, E., Man, A. L., Le Gall, G., Branca, J. J., Ghelardini, C., Amedei, A., Bertelli, E., & Regoli, M. (2020). Faecal microbiota transplant from aged donor mice affects spatial learning and memory via modulating hippocampal synaptic plasticity-and neurotransmission-related proteins in young recipients. *Microbiome, 8*(1), 1-19.

Dalla Nora, M., Hörner, R., De Carli, D. M., ROCHA, M. P. d., ARAUJO, A. F. d., & Fagundes, R. B. (2016). Is the immunocromatographic fecal antigen test effective for primary diagnosis of Helicobacter pylori infection in dyspeptic patients? *Arquivos de Gastroenterologia, 53*, 224-227.

Davar, D., Dzutsev, A. K., McCulloch, J. A., Rodrigues, R. R., Chauvin, J.-M., Morrison, R. M., Deblasio, R. N., Menna, C., Ding, Q., & Pagliano, O. (2021). Fecal microbiota transplant overcomes resistance to anti–PD-1 therapy in melanoma patients. *Science, 371*(6529), 595-602.

de Martel, C., Georges, D., Bray, F., Ferlay, J., & Clifford, G. M. (2020). Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. *The Lancet Global Health, 8*(2), e180-e190.

Ding, S.-Z. (2020). Global whole family based-Helicobacter pylori eradication strategy to prevent its related diseases and gastric cancer. *World journal of gastroenterology, 26*(10), 995.

Dore, M. P., & Pes, G. M. (2021). What is new in

Helicobacter pylori diagnosis. An overview. *Journal of Clinical Medicine, 10*(10), 2091.

Ferwana, M., Abdulmajeed, I., Alhajiahmed, A., Madani, W., Firwana, B., Hasan, R., Altayar, O., Limburg, P. J., Murad, M. H., & Knawy, B. (2015). Accuracy of urea breath test in Helicobacter pylori infection: meta-analysis. *World journal of gastroenterology: WJG, 21*(4), 1305.

Ford, A. C., Yuan, Y., & Moayyedi, P. (2020). Helicobacter pylori eradication therapy to prevent gastric cancer: systematic review and meta-analysis. *Gut, 69*(12), 2113-2121.

Fumet, J.-D., Richard, C., Ledys, F., Klopfenstein, Q., Joubert, P., Routy, B., Truntzer, C., Gagné, A., Hamel, M.-A., & Guimaraes, C. F. (2018). Prognostic and predictive role of CD8 and PD-L1 determination in lung tumor tissue of patients under anti-PD-1 therapy. *British journal of cancer, 119*(8), 950-960.

Godbole, G., Mégraud, F., & Bessède, E. (2020). Diagnosis of Helicobacter pylori infection. *Helicobacter, 25*, e12735.

Graham, D. Y. (2020). Transitioning of Helicobacter pylori therapy from trial and error to antimicrobial stewardship. *Antibiotics, 9*(10), 671.

Hathroubi, S., Hu, S., & Ottemann, K. M. (2020). Genetic requirements and transcriptomics of Helicobacter pylori biofilm formation on abiotic and biotic surfaces. *npj Biofilms and Microbiomes, 6*(1), 56.

Hooi, J. K., Lai, W. Y., Ng, W. K., Suen, M. M., Underwood, F. E., Tanyingoh, D., Malfertheiner, P., Graham, D. Y., Wong, V. W., & Wu, J. C. (2017). Global prevalence of Helicobacter pylori infection: systematic review and meta-analysis. *Gastroenterology, 153*(2), 420-429.

Htun, N. S. N., Odermatt, P., Müller, I., Yap, P., Steinmann, P., Schindler, C., Gerber, M., Du Randt, R., Walter, C., & Pühse, U. (2018). Association between gastrointestinal tract infections and glycated hemoglobin in school children of poor neighborhoods in Port Elizabeth, South Africa. *PLoS neglected tropical diseases, 12*(3), e0006332.

Ierardi, E., Losurdo, G., Mileti, A., Paolillo, R., Giorgio, F., Principi, M., & Di Leo, A. (2020). The puzzle of coccoid forms of Helicobacter pylori: beyond basic science. *Antibiotics, 9*(6), 293.

Jaka, H., Rhee, J. A., Östlundh, L., Smart, L., Peck, R., Mueller, A., Kasang, C., & Mshana, S. E. (2018). The magnitude of antibiotic resistance to Helicobacter pylori in Africa and identified mutations which confer resistance to antibiotics: systematic review and meta-analysis. *BMC Infectious Diseases, 18*(1), 1-10.

Keller, J., Hammer, H. F., Afolabi, P. R., Benninga, M., Borrelli, O., Dominguez-Munoz, E., Dumitrascu, D., Goetze, O., Haas, S. L., & Hauser, B. (2021). European guideline on indications, performance and clinical impact of 13C-breath tests in adult and pediatric patients: an EAGEN, ESNM, and ESPGHAN consensus, supported by EPC. *UEG Journal, 9*(5), 598-625.

Kim, J., & Wang, T. C. (2021). Helicobacter pylori and gastric cancer. *Gastrointestinal Endoscopy Clinics, 31*(3), 451-465.

Lee, Y.-C., Chiang, T.-H., Chiu, H.-M., Wu, M.-S., Yeh, Y.-P., Chen, T. H.-H., Chen, S. L.-S., Yen, A. M.-F., Fann, J. C.-Y., & Chiu, S. Y.-H. (2021). Community-based gastric cancer screening coupled with a National Colorectal Cancer Screening Program: baseline results. *Gastroenterology, 160*(6), 2159-2161. e2154.

Leszczyńska, K., Namiot, A., Namiot, Z., Leszczyńska, J., Jakoniuk, P., Chilewicz, M., Namiot, D., Kemona, A., Milewski, R., & Bucki, R. (2010). Patient factors affecting culture of Helicobacter pylori isolated from gastric mucosal specimens. *Advances in medical sciences, 55*(2), 161-166.

Makristathis, A., Hirschl, A. M., Mégraud, F., & Bessède, E. (2019). Diagnosis of Helicobacter pylori infection. *Helicobacter, 24*, e12641.

Malfertheiner, P., Megraud, F., O'morain, C., Gisbert, J., Kuipers, E., Axon, A., Bazzoli, F., Gasbarrini, A., Atherton, J., & Graham, D. Y. (2017). Management of Helicobacter pylori infection—the Maastricht V/Florence consensus report. *Gut, 66*(1), 6-30.

Mezmale, L., Coelho, L. G., Bordin, D., & Leja, M. (2020). Epidemiology of Helicobacter pylori. *Helicobacter, 25*, e12734.

Mišak, Z., & Hojsak, I. (2021). Helicobacter Pylori Gastritis and Peptic Ulcer Disease. In Textbook of Pediatric Gastroenterology, Hepatology and Nutrition: A Comprehensive Guide to Practice (pp. 169-184). Springer.

Nakashima, H., Kawahira, H., Kawachi, H., & Sakaki, N. (2018). Artificial intelligence diagnosis of Helicobacter pylori infection using blue laser imaging-bright and linked color imaging: a single-center prospective study. *Annals of gastroenterology, 31*(4), 462.

Nezami, B. G., Jani, M., Alouani, D., Rhoads, D. D., & Sadri, N. (2019). Helicobacter pylori mutations detected by next-generation sequencing in formalin-fixed, paraffin-embedded gastric biopsy specimens are associated with treatment failure. *Journal of clinical microbiology, 57*(7), 10.1128/jcm. 01834-01818.

Nyssen, O. P., Vaira, D., Tepes, B., Kupcinskas, L., Bordin, D., Pérez-Aisa, Á., Gasbarrini, A., Castro-Fernández, M., Bujanda, L., & Garre, A. (2022). Room for improvement in the treatment of Helicobacter pylori infection: lessons from the European Registry on *H. pylori* management (Hp-EuReg). *Journal of clinical gastroenterology, 56*(2), e98-e108.

Park, J. S., Jun, J. S., Seo, J. H., Youn, H. S., & Rhee, K. H. (2021). Changing prevalence of Helicobacter pylori infection in children and adolescents. *Clin Exp Pediatr, 64*(1), 21-25. https://doi.org/10.3345/cep.2019.01543

Pich, J. (2019). Noninvasive diagnostic tests for helicobacter pylori infection. *Gastroenterology Nursing, 42*(1), 101-102.

Poddar, U. (2019). Helicobacter pylori: a perspective in

low-and middle-income countries. *Paediatrics and International Child Health, 39*(1), 13-17.

Pohl, D., Keller, P. M., Bordier, V., & Wagner, K. (2019). Review of current diagnostic methods and advances in Helicobacter pylori diagnostics in the era of next generation sequencing. *World journal of gastroenterology, 25*(32), 4629.

Sonnenberg, A., Turner, K. O., & Genta, R. M. (2020). Low prevalence of Helicobacter pylori-positive peptic ulcers in private outpatient endoscopy centers in the United States. *Official journal of the American College of Gastroenterology | ACG, 115*(2), 244-250.

Stefano, K., Marco, M., Federica, G., Laura, B., Barbara, B., Gioacchino, L., & Gian, L. d. A. (2018). Helicobacter pylori, transmission routes and recurrence of infection: state of the art. *Acta Bio Medica: Atenei Parmensis, 89*(Suppl 8), 72.

Stefano, K., Rosalia, A., Chiara, B., Federica, G., Marco, M., Gioacchino, L., Fabiola, F., & Gian, L. d. A. (2018). Non-invasive tests for the diagnosis of helicobacter pylori: state of the art. Acta Bio Medica: *Atenei Parmensis, 89*(Suppl 8), 58.

Szymczak, A., Ferenc, S., Majewska, J., Miernikiewicz, P., Gnus, J., Witkiewicz, W., & Dąbrowska, K. (2020). Application of 16S rRNA gene sequencing in Helicobacter pylori detection. PeerJ, 8, e9099.

Tonkic, A., Tonkic, M., Lehours, P., & Mégraud, F. (2012). Epidemiology and Diagnosis of H elicobacter pylori Infection. *Helicobacter, 17*, 1-8.

Tonkic, A., Vukovic, J., Vrebalov Cindro, P., Pesutic Pisac, V., & Tonkic, M. (2018). Diagnosis of Helicobacter pylori infection: A short review. *Wiener klinische Wochenschrift, 130*, 530-534.

Uribe-Herranz, M., Bittinger, K., Rafail, S., Guedan, S., Pierini, S., Tanes, C., Ganetsky, A., Morgan, M. A., Gill, S., & Tanyi, J. L. (2018). Gut microbiota modulates adoptive cell therapy via CD8α dendritic cells and IL-12. *JCI insight, 3*(4).

van Wijck, Y., de Kleijn, S., John-Schuster, G., Mertens, T. C., Hiemstra, P. S., Müller, A., Smits, H. H., & Taube, C. (2018). Therapeutic application of an extract of Helicobacter pylori ameliorates the development of allergic airway disease. *The Journal of Immunology, 200*(5), 1570-1579.

Wilson, B. E., Routy, B., Nagrial, A., & Chin, V. T. (2020). The effect of antibiotics on clinical outcomes in immune-checkpoint blockade: a systematic review and meta-analysis of observational studies. *Cancer Immunology, Immunotherapy, 69*, 343-354.

Zamani, M., Ebrahimtabar, F., Zamani, V., Miller, W., Alizadeh-Navaei, R., Shokri-Shirvani, J., & Derakhshan, M. (2018). Systematic review with meta-analysis: the worldwide prevalence of Helicobacter pylori infection. *Alimentary pharmacology & therapeutics, 47*(7), 868-876.

Zhao, J. B., Yuan, L., Yu, X. C., Shao, Q. Q., Ma, J., Yu, M., Wu, Y., Qi, Y. B., Hu, R. B., & Wei, P. R. (2021). Whole family—based Helicobacter pylori eradication is a superior strategy to single-infected patient treatment approach: A systematic review and meta-analysis. *Helicobacter, 26*(3), e12793.